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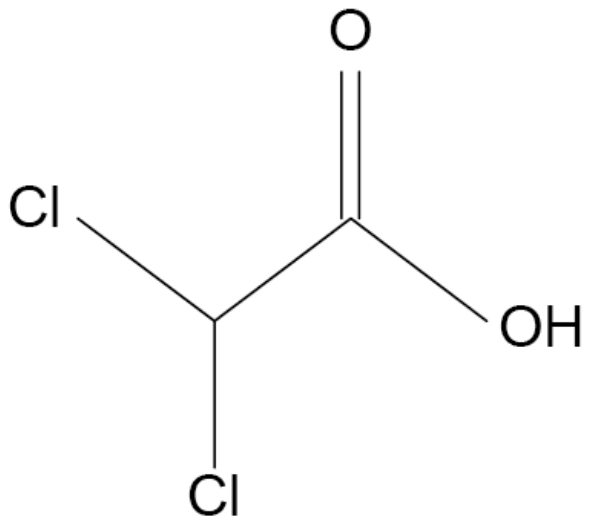
Toxicology and Carcinogenicity Studies of Dichloroacetic Acid



DICHLOROACETIC ACID

CAS No. 79-43-6

Chemical Formula: $C_2H_2Cl_2O_2$ Molecular Weight: 128.9426

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Nomination and Selection

- **Nomination:**
  - Dichloroacetic acid (DCA) nominated by EPA and NIEHS
- **Rationale:**
  - Part of a larger study to evaluate representative disinfection byproducts (DBPs) found in municipal drinking water. DCA is a well studied and well known rodent carcinogen.
  - Genetically modified mouse models may be more rapid, use fewer animals and provide more mechanistic insights into carcinogenic responses. They could prove especially useful for evaluating chemicals that appear in drinking water.

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DBP Selection

Dichloroacetic acid (DCA)

- DCA is well studied rodent liver carcinogen
- Representative haloacetic acid second most common DBP
- Weakly mutagenic

Bromodichloromethane (BDCM)

- BDCM is well studied rodent carcinogen (colon, kidney - rats, kidney - male mice, liver - female mice).
- Representative trihalomethane
- Mutagenic in bacterial systems, does not induce micronuclei

Bromate

- Common anion found after ozonation of water
- Well studied renal carcinogen in rodents, mutagenic

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Genetically Modified Mouse Models

- **NTP evaluating two GMM Models**
  - Tg.AC is reported to respond to mutagens/nonmutagens
    - Dermal and other routes of exposure
    - Papillomas often reporter phenotype
  - p53 haploinsufficient mouse reported to respond to mutagens
    - Feed, water, inhalation routes of exposure
    - Systemic tumors often reporter phenotype
  - Evaluated these GMM models versus standard rodent models for 3 DBPs
  - Evaluated the GMM Models at 6 months and 9 months

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DCA Study Design

- **Dermal studies**
  - Male and female Tg.AC hemizygous mice
  - 6- and 9-month[a] studies (N = 15 & 10)
  - Dose groups - 0, 31, 125, or 500 mg/kg

- **Drinking water studies**
  - Male & female Tg.AC hemizygous mice & p53 haploinsufficient mice
  - 6- and 9-month[a] studies (N = 15 & 10)
  - Dose groups - 0, 500, 1000, or 2000 mg/L

[a]Dermal exposures = 26 & 39 weeks

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Results of Dermal Studies in Tg.AC Mice

- Survival and body weight were generally similar to controls
  - Body weights of treated females higher at 6 and 9 months
  - Body weights of treated males lower at 9 months
- Minimal nonneoplastic dermal changes
  - Hyperkeratosis increased at 6 and 9 months
  - Hyperplasia increased at 6 and 9 months
- Increased dermal papillomas in 9 month study

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Dermal Lesions - Site of Application Tg.AC Mice

Dose (mg/kg)	0	31	125	500
Male				
Hyperplasia				
6-month[a]	0	13% (1.0)	73%* (1.0)	87%**(1.8)
9-month[b]	0	0	80%** (1.3)	90%** (2.2)
Papillomas (all)				
6-month	0	0	7%	13%
9-month	0	0	20%	80%**
Female				
Hyperplasia				
6- month [a]	0	7% (1.0)	67%** (1.0)	87%** (1.8)
9- month [b]	0	0	30% (1.3)	60%** (1.3)
Papillomas (all)				
6- month	0	0	0	13%
9- month	0	0	0	60%**

[a]N=15
[b]N=10
[c]Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked.
*P≤0.05, **P≤0.01

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DCA Study Design

- Dermal studies
  - Male and female Tg.AC hemizygous mice
  - 6- and 9-month[a] studies (N = 15 & 10)
  - Dose groups - 0, 31, 125, or 500 mg/kg
- Drinking water studies
  - Male & female Tg.AC hemizygous mice & p53 haploinsufficient mice
  - 6- and 9-month[a] studies (N = 15 & 10)
  - Dose groups - 0, 500, 1000, or 2000 mg/L
    - Approximately 75, 145 and 235 mg/kg to males
    - Approximately 100, 185 and 280 mg/kg to females

[a]Drinking water exposures = 26 & 41 weeks

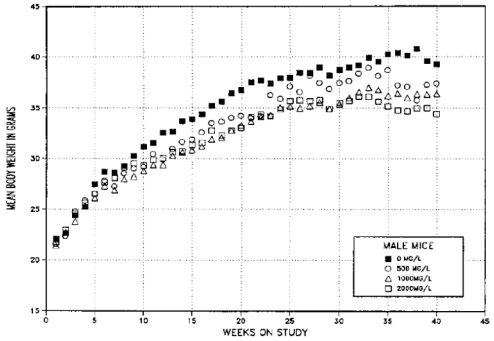
Survival and Body Weights in the DCA Tg. AC Drinking Water Studies

6-month Studies

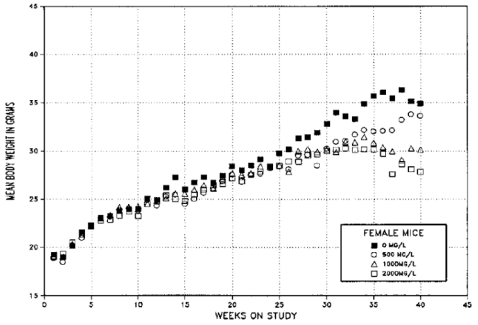
- Males — No effect survival; increased body weight
- Females — Decreased survival; decreased body weight

9-month Studies

- Males — No effect survival; decreased body weight
- Females — No effect survival; decreased body weight



MALES 9 mo.



FEMALES 9 mo.

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Hepatocyte vacuolization - DW Tg.AC Study

Dose (mg/L)	0	500	1000	2000
Male				
Vacuolization				
6-month[a]	47% (1.0)	87%* (1.8)[c]	100%** (2.7)	100%** (3.7)
9-month[b]	90% (2.0)	100% (2.3)	90% (3.2)	100% (3.8)
Female				
Vacuolization				
6-month[a]	40% (1.3)	67% (2.7)	93%** (3.1)	93%** (3.7)
9-month[b]	70% (2.0)	90% (2.6)	90% (2.9)	100% (3.0)

[a]N=15
[b]N=10
[c]Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked,
*P≤0.05, **P≤0.01

Alveolar/Bronchiolar Tumors - Tg.AC Mice

Concentration (mg/L) in DW	0	500	1000	2000
Males (75, 145, 235 mg/kg)				
6-month[a]	0	0	**7%**	0
9-month[b]	10%[c]	20%	70%**	30%
Females (100, 185, 280mg/kg)				
6-month[a]	0	**7%**	0	**7%**
9-month[b]	0	0	0	20%
Dermal Study (mg/kg)	0	31	125	500
Males				
6-month[a]	7%	0	7%	0
9-month[b]	0	0	10%	20%
Females				
6-month[a]	0	7%	7%	0
9-**month**[b]	0	0	10%	20%

[a]N=15
[b]N=10
[c]Adenomas except carcinomas noted in **bold**
**P≤0.01.
Historical rates of pulmonary adenomas are 4/112 (3.6%) in male Tg.AC mice at 39-43 weeks.
In these same ten studies pulmonary carcinomas were not found.

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DCA Tg.AC mouse Conclusions

Dermal Study

Increased incidence of squamous cell papillomas at site of application in mice at 500 mg/kg DCA at 9 months

Hyperplasia and hyperkeratosis increased at 6 and 9 months

Drinking Water Study

Increased incidence of alveolar/bronchiolar adenomas in male mice exposed to 1000 mg/L for 9 months

Increased incidences and/or severities of hepatocyte cytoplasmic vacuolization both males/females at 6 or 9 months

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Study Summary p53 Mouse Model

6- and 9-month[a] Drinking Water Studies

Concentration in water: 0, 500, 1000, 2000 mg/L

Same concentrations as Tg.AC study

Same concentrations are carcinogenic in rodents

[a]Six and nine months = 26 & 41 weeks

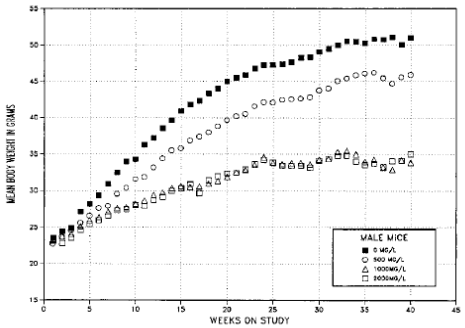
Survival and Body Weights in the DCA p53 Drinking Water Studies

6-month Drinking Water Studies

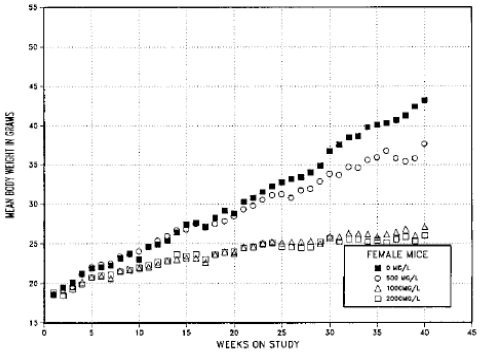
- Males No effect survival; decreased body weight
- Females No effect survival; decreased body weight

9-month Drinking Water Studies

- Males No effect survival; decreased body weight
- Females No effect survival; decreased body weight



MALES 9 mo.



FEMALES 9 mo.

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Hepatocyte Vacuolization in p53 DW Study

Dose (mg/L)	0	500	1000	2000
Male				
Vacuolization				
6-month[a]	100% (2.7) [c]	100% (3.4)	100% (3.4)	100% (4.0)
9-month[b]	90% (3.6)	100% (3.0)	100% (3.7)	100% (3.8)
Female				
Vacuolization				
6-month[a]	20% (1.0)	100%**(2.2)	100%** (3.1)	100%** (3.5)
9-month[b]	100% (1.9)	100% (2.7)	100% (3.7)	100% (3.6)

[a]N=15
[b]N=10
[c]Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked,
**P≤0.01

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DCA Conclusions

Male and Female p53 haploinsufficient mice:

No evidence of carcinogenic activity:

- 0, 500, 1000 and 2000 mg/L in drinking water
- Exposures for 6 and 9 months.

The incidence and/or severities of cytoplasmic vacuolization of the hepatocytes were increased in both males and females exposed to DCA in the drinking water for 6 or 9 months

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Conclusions on DCA in GMM Models

1) Six month exposure insufficient to detect a carcinogenic effect in either Tg.AC or p53 Mice

1) Six months may not be sufficient time
2) Increasing group size beyond 15 mice may increase sensitivity

2) Weakly mutagenic but potent hepatocarcinogen failed to cause cancer in p53 model

1) Decreased body weight suggest higher dose not possible
2) Nonneoplastic response in liver suggest target organ affected

3) Weakly mutagenic but potent hepatocarcinogen caused weak dermal response in Tg.AC mice

1) Dermal papillomas significant in high dose only and 9 months only
2) Lung tumors in drinking water study considered to be related to DCA exposure but significantly increased in mid-dose males only

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NTP Technical Reports Review Subcommittee Meeting

Dichloroacetic acid

GMM 11